Comparative Assessment of Gingival Microleakage between Vitrebond resin Modified Glass Ionomer Cement Liner Versus Biodentine Liner in Simulated Open Sandwich Class II Composite Resin Restorations Using Confocal Microscopy.

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ABSTRACT

Background: To study and compare gingival microleakage between two lining materials; Vitrebond (RMGIC) and Biodentine which one will give least microleakage in ClassII nanohybrid composite restorations using open sandwich technique using confocal microscopy. Methods: Standardized Class II proximoocclusal cavity preparations were prepared in permanent premolars. Teeth were restored using two lining materials (n=15): 3M ESPE; Vitrebond Light Cure Resin Modified GIC and Septodont; Biodentine followed by nanohybrid composite resin is done using open sandwich technique. The restorations were subjected to thermocycling procedure (x2000 5C–55C 10 s/min) in hot and cold. Sealing of apices with sticky wax, coating with nail varnish and soaked in freshly prepared 0.25% Rhodamine B fluorescent dye for 24 h at 37°C. Teeth were sectioned longitudinally in mesiodistal direction under continuous cooling into three slabs of 1 mm thickness and studied under a confocal microscope for dye penetration. Statistical Analysis: Data was evaluated by using One-way analysis of variance, Post Hoc multiple comparison test and the Tukey's multiple comparison test employing 95% (p = 0.05). Results: Biodentine and Vitrebond (RMGIC) produced the minimum microleakage scores with no significant differences between them. Biodentine showed minimum leakage with more continuous margins. Conclusion: Biodentine and Vitrebond light cure resin modified glass ionomer cement when used as a lining material under nanohybrid composite restorations in open sandwich technique gave satisfactory least microleakage scores.

Keywords: Nanohybrid composite, Class II cavities, Vitrebond Resin modified glass ionomer cement, Biodentine, Open sandwich technique.

INTRODUCTION

Microleakage at the tooth/restoration interface is considered to be a major factor influencing the longevity of the restoration. [11] Satisfactory esthetics and longevity have been reported in smaller restorations but in larger restorations, long-term success is more difficult to attain. Poor marginal adaptation and considerable microleakage have been shown in cavities with the cervical margin located at or below the cervico-enamel junction is a matter of concern to the clinician. [2,3] Microleakage

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can act as a seed sower for the secondary caries to develop and gradually results in hypersensitivity of the restored teeth, discoloration, recurrent caries, injury leading to the failure of and pulpal restoration. So an impervious sealing of the cavity is utmost mandatory for durable restoration.^[4] Interest in Esthetic dentistry has resulted in composite resin restoration being increasingly used not only as a replacement material for failed or unesthetic amalgams but also as a first choice to restore anterior as well as posterior teeth.^[5] Composite resins are popular worldwide because of their excellent esthetic value and needing minimal tooth preparation due to micromechanical bonding to tooth structure. It became possible only after progressive research in composition and curing methodology of the composite restorative Thus materials. newer modifications microfilled and hybrid composites have evolved. [6]

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Recently developed nanofill composites containing extremely small filler particles $(0.005\text{-}0.01\mu\text{m})$ resulting in esthetic properties similar to those of microfills while maintaining physical properties equivalent to those of hybrid composites. This allows the clinician to use them both for anterior as well as for posterior restorations. $^{[6]}$

Inspite of all the advantages, we have not yet been able to overcome volumetric polymerization shrinkage (1-5% volume) that occurs upon curing and produces stress between bonded restorations and tooth walls which in turn produces powerful forces that can separate restoration from tooth resulting in gap formation more so at cervical margins. This is due to lack of enamel at cement enamel junction and incomplete adhesion between and composite restoration. microleakage is one of the major reasons for failure Class II restorations restored composites.^[7,8] In an effort to reduce the side effects caused by polymerization shrinkage stress, Mclean and Wilson (1997) suggested sandwich technique in which an intermediary layer between composite restoration and tooth is placed that acts as a stress breaker because of low flexural strength (similar to dentine) resulting in reduction of gap produced polymerization formation by shrinkage.[9,10] This layer acts as a buffer and thus decreases microleakage. Various materials like GIC, flowable composites, self or dual-cured composite resin material, resin modified glass ionomer cement, biodentine have been used in sandwich technique as stress absorbing intermediary layer.

Vitrebond light cured resin modified glass ionomer cement (RMGIC) is preferred because of better marginal marginal adaptation due to its chemical adhesion and low polymerization shrinkage and thus reduces microleakage. [5,11] Biodentine is biocompatible, bioactive material having outstanding sealing and mechanical properties sufficient to withstand occlusal load when protected with composite resin material. It has been advocated as a dentine substitute in sandwich restoration, leading to decrease in microleakage. [12] Microleakage is commonly assessed in vitro using

dye penetration like rhodamine B using 3D-confocal laser scanning microscopy for increasing optical resolution and visualizing tooth surface features. It gives clear indication of leakage of dye due to its lens focus that occur some microns beneath the observed surface. This is one of the best methods to detect microleakage. [8]

The purpose of this in-vitro study is to compare gingival microleakage with two different lining materials used in open sandwich technique for Class II composite restorations under confocal microscopy.

MATERIALS AND METHODS

In this study, Nanohybrid composite resin, 3M ESPE; Vitrebond Light Cure Resin Modified GIC and Septodont; Biodentine were evaluated. Names, codes, manufacturers, mixing, and application procedures of the materials tested were also summarized. Non carious freshly extracted forty five intact human maxillary premolars of approximately same dimensions were collected, cleaned with slurry of pumice and stored in 1% chloramine-T solution (Himedia Labs., India) until use. Standardized Class II cavities were made involving the proximal and occlusal surfaces using No. 245 and 169 L tungsten carbide burs in a high speed air rotor with water spray with dimensions: Pulpal Depth: 2.5mm, Bucco-Lingual Width: 2mm. Axial Wall Height: 3mm till CEJ, Gingival Seat: 0.8 mm into dentin. The teeth were randomly divided into three experimental groups (n=15). GROUP 1- (CONTROL) Bonding (ScotchbondTM Universal Adhesive 3M ESPE) was applied on 15 Class II cavities with a microbrush for 35 sec, thinned by mild air pressure for 5 sec, and then polymerized for 20 sec. In each sample tofflemire metal matrix band and wedge was applied and restored with FiltekTM Z250 XT Nanohybrid composite (3M ESPE) in increments gingivo-occlusally (each increment being 2 mm). Each layer or increment was polymerized for 40 sec from the occlusal surface with a LED light curing unit (Celalux 2 High-Power LED curinglight, Voco GmbH, Cuxhaven, Germany) at a light intensity of 1000 mW/cm2 according to manufacturers' instructions. Bonding (ScotchbondTM Universal Adhesive 3M ESPE) was applied on 15 Class II cavities with a microbrush according to the manufacturer's instructions, especially on enamel edges. It was applied for 35 sec, thinned by mild air pressure for 5 sec, and then polymerized for 20 sec. GROUP II-15 Class II cavities were restored with an intermediary layer of Vitrebond Light Cure RMGIC (3M ESPE) in a horizontal increments in the gingival half of the proximal box (Open Sandwich Technique). It was polymerized for 20 seconds. The rest of the cavity was then restored with nanohybrid composite resin in an incremental manner as described for Group I. GROUP III-Biodentine (Septodont, Saint-Maur-des-fosses Cedex, France) was mixed according manufacturer's recommendations. An intermediary layer of Biodentine was placed in 15 Class II cavities in the gingival half of the proximal box (Open Sandwich Technique). After 12 minutes the prepared cavity was restored with nanohybrid composite resin in an incremental manner as described for Group I. The finishing and polishing of all restorations were achieved with the sequential use of finishing and polishing burs, Diamond or Aluminum oxide discs, rubber cups, wheels and pastes. Proximal margins were finished

with Sof-Lex discs (3M ESPE). The teeth were stored in isotonic saline solution at 37°C water bath for 24 h. The samples were subjected to thermocycling in customized thermocycling device i.e. 2000 thermal cycles at 5°C, 37°C and 55°C in water with a dwell time of 30 sec and transfer time of 10 seconds. Apices of all the teeth will then be sealed with sticky wax and two coats of Nail varnish will be applied on the entire sample surface except for the restoration and 1mm area beyond the margins of restoration. Further the samples will be soaked in freshly prepared 0.25% Rhodamine B Fluorescent dye for 24 hours at 37°C after which they will be thoroughly rinsed under tap water. Samples will then be sectioned in Mesio-Distal dimension from the centre of the restoration with diamond coated disc mounted in straight hand piece under continuous cooling into three slabs of 1 mm thickness and studied under a confocal microscope for dye penetration at 10 X magnification. [Figure 1]

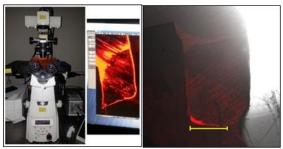


Figure 1: Sections seen under confocal microscope showing dye leakage at the gingival wall

Depth of microleakage was noted & tabulated. Dye penetration was assessed according to the scoring criteria given by Leevaloj C, Cochran MA et al.

Table 1: Scoring Criteria

Score Description

No evidence of dye penetration at tooth restoration interface

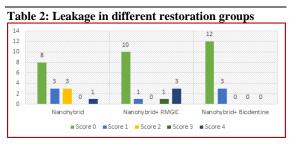
Dye penetration along the cavity wall upto 1/3rd of cavity depth

Dye penetration greater than 1/3rd, but less than 2/3rd of cavity depth.

Dye penetration greater than 2/3rd of cavity depth but not along the dentinal tubules

Dye penetration to the cavity depth and along the

dentinal tubules.

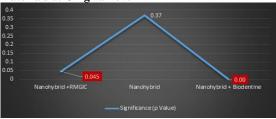


Depth of penetration was evaluated using NIS-Elements D (Advance Research Microscope Imaging Software) BR Ver4.13.05 32 bit edition. The tabulated results of microleakage were then put to statistical analysis using One-way ANOVA, Post Hoc multiple comparison test and t Test.

RESULTS

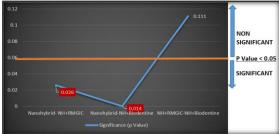
On application of Analysis of Variance (ANOVA) carried out between Vitrebond (RMGIC) and Biodentine at the gingival level. The analysis revealed significant differences between Nanohybrid + Biodentine and Nanohybrid + Vitrebond. Further, after applying a post-hoc test [Fisher's LSD (Least Significant Difference)], it was revealed that the material causing the least microleakage are ranked according to superiortity is NH+Biodentine > NH+RMGIC > Nanohybrid.

Table 3: Significance (p) values of ANOVA among materials at Gingival level



On application of paired samples t-test among the various materials used at the gingival level, a significant difference was seen between Nanohybrid and Nanohybrid + Vitrebond RMGIC restorative material (p=0.026) and between Nanohybrid and Nanohybrid + Biodentine (p=0.14). The results indicate better prevention of marginal leakage among Vitrebond RMGIC and Biodentine when used with Nanohybrid Composite.

Table 4: Application of paired samples t-test used at the Gingival level



DISCUSSION

Many changes happened in the field of dentistry in the last decade. In recent years, preventive methods, minimally invasive methods and longevity of restorations have gained special attention in restorative dentistry. The fact that composite restorations exhibit leakage at marginal interfaces with tooth structure has no surprise to the dentists. It has been known that conventional composite restorative materials do not provide a complete hermetic seal and various studies have demonstrated that the leakage of fluid occur between the restoration and the prepared tooth surface. This gingival leakage has been implicated as an etiological factor in the

inflammation of the dental pulp following the insertion of composite restorations. [15] However, a number of problems are associated while using dental composites, with the primary ones being polymerization shrinkage, moisture sensitivity, and lack of essential bonding to enamel and dentin. [16] A liner must act as a barrier to protect the dental pulpal complex and induce the formation of new dentine bridge or dentine like bridge between the pulp and restorative material.

In this study, two new materials that might potentially provide the necessary properties of lining material, Biodentine and Vitrebond RMGIC, were compared with nanohybrid resin with regard to its gingival microleakage using Rhodamine B fluorescent dye and Confocal Laser Scanning microscope.

Different leakage tracer dyes are used to assess microleakage such as rhodamine B, methylene blue, indiana ink, eosin, erythrosine, crystal violet, silver nitrate basic fuchsin and nickel sulphate have been recommended to test the sealing properties of restorative materials both in vivo and in vitro. [17] For the evaluation of the interface between human dentin and Biodentine/Vitrebond/Nanohybrid, we used 3-D confocal reflection to observe interface micropermeability as it offers a number of advantages over other techniques which include:

- 1. Non-destructive examination of the samples
- Non- dehydrated samples can be used: Drying of samples, which is indispensable for conventional SEM or TEM, is not necessary with CLSM, leading to a decreased risk of shrinking or other drying artifacts
- 3. No specific sectioning technique required: This decreases the possibility of artifacts produced during the preparation of the specimens.^[18] BiodentineTM has been shown to be biocompatible, i.e. it does not damage pulpal cells in vitro or in vivo, and is capable of stimulating tertiary dentin formation.^[19] Raskin A, et al (2012),^[20] found that biodentine may be used as a dentine substitute in cervical approximal cavities or as a bulk provisional restoration where the cervical extent is under the CEJ. Biodentine a tricalcium silicate, due its good sealing properties, high compression strengths and short setting time has a potential of good restorative material. The good marginal integrity of open sandwich restorations filled with Biodentine is likely due to the outstanding ability of the calcium silicate materials to form hydroxyapatite crystals at the surface which contribute to the sealing efficiency of the material. Studies have advocated the use of BiodentineTM as dentine substitute. Dejou J et al (2015) evaluated the micro leakage resistance of BiodentineTM in comparison with one of the best sealing systems, resin modified glass ionomers (Fuji II LC, GC Corp.) shows biodentineTM exhibited better leakage

resistance both to enamel and to dentine compared to Fuji II LC, RMGIC.^[19]

Another type of liner used that can be used is resin modified glass ionomer liner, in an 'open sandwich' technique. Placement of resin modified glass ionomer cement liner also reduced the total volume of composite resin, thus reducing the amount of shrinkage and stresses associated with it.[21] The cement can provide an anticariogenic effect via fluoride release. RMGIC can be photocured, showed early resistance to moisture contamination, is easy to place, sets on command, and bonds chemically to composite resin. Extension of conventional GIC to the external cavosurface margin resulted in severe degradation. However, it is now possible to extend RMGIC to external cavosurface (open sandwich) compared to maintaining it short of the margin (closed sandwich).[22] It has been shown that the inability of conventional GICs to produce an effective seal depends on two factors: 1) the material's sensitivity to moisture during placement and the early set 2) the dehydration after setting, resulting in crazing and cracking.^[23] Wilson and Kent in (1971),^[24] stated that RMGIC is used as a liner in resin composite and amalgam restorations with the objective of protecting the restored tooth against thermal and chemical trauma and also of keeping the cavity margin sealed.

Based on nanotechnology, nanocomposites were introduced which have been improved to overcome the drawbacks of recurrent caries, post-operative sensitivity, enamel fracture, marginal staining, microleakage and eventual failure of restorations. According to the studies conducted by Min-Huey Chena et al (2006) and Mui S. Soh et al (2007), the polymerization shrinkage of the nanocomposites is less than that of the conventional resin composite restorative materials.^[25]

The results of the present study showed that maximum microleakage scores were seen in Group 1; Nanohybrid (Control) as compare to Group II; Vitrebond (RMGIC) and Group III; Biodentine. However, Biodentine exhibits least leakage score as compare to Vitrebond and Nanohybrid.

In this study when two materials were compared to check microleakage at the gingival level, a significant difference in microleakage values between Nanohybrid and Nanohybrid + Vitrebond (p=0.026) restorative material and between Nanohybrid and Nanohybrid + Biodentine (p=0.014) was found. The results indicate better prevention of marginal leakage among Vitrebond and Biodentine when used with Nanohybrid Composite. Also, there is no significant difference found in Group II; Vitrebond (RMGIC) and Group III; Biodentine microleakage values when seen under confocal microscopy.

When all the materials are compared individually at gingival level, there is significant difference found

in Group I (p=0.111) as compared to Group II (p=0.045) and Group III; Biodentine (p=0.000) but no significance in Group II and Group III.

It was revealed that the material causing the least microleakage are ranked according to superiortity is Biodentine > Vitrebond; RMGIC > Nanohybrid.

As our study was in vitro study and in order to achieve a valid comparison, the experimental protocol must reproduce optimal clinical conditions. Thus, for clinical relevance, further in vivo studies with a larger sample size are required.

CONCLUSION

Within the limitations of the present in vitro study, it can be concluded that:

- Both Biodentine and Vitrebond RMGIC were more effective in sealing the gingival margins of Class II open sandwich restorations as compared to Nanohybrid composite resin.
- Recent calcium silicate cement i.e. Biodentine turns to be the best for sealing Class II gingival margins than rest of the groups and showed minimum microleakage.
- 3. No material was able to totally eliminate microleakage in Class II restorations with gingival margin ended in dentine.

A definite conclusion as to which material should be preferred between Vitrebond (RMGIC) and Biodentine can be withdrawn after correlating the findings of the present study with clinical research. Therefore more studies are advocated.

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